

Figure 1. Sequence and structure of 27mer RNA target

105190" 27E4886D

Docket No.: IBIS-0369  
Title: MASS SPECTROMETRIC METHODS FOR  
BIOMOLECULAR SCREENING  
Inventors: Stanley T. Crooke, Richard Griffey and  
Steven Hofstadler  
Atty: Paul K. Legaard - Telephone: 215 568 3100  
Sheet 2 of 33

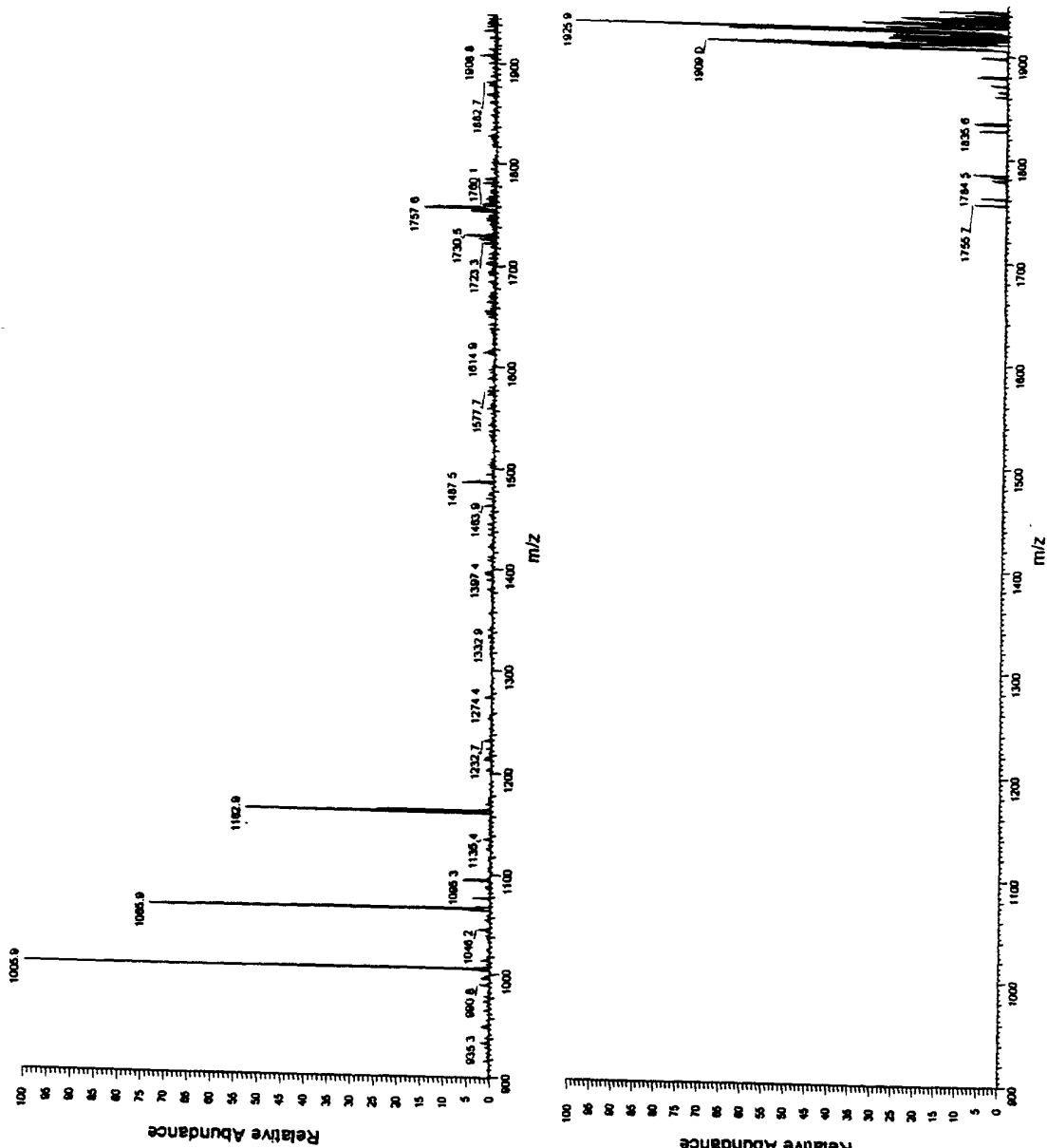


Figure 2. MS/MS of control RNA/DNA (upper); control+paromomycin (lower)

706790" 47548860

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Sheet 3 of 33

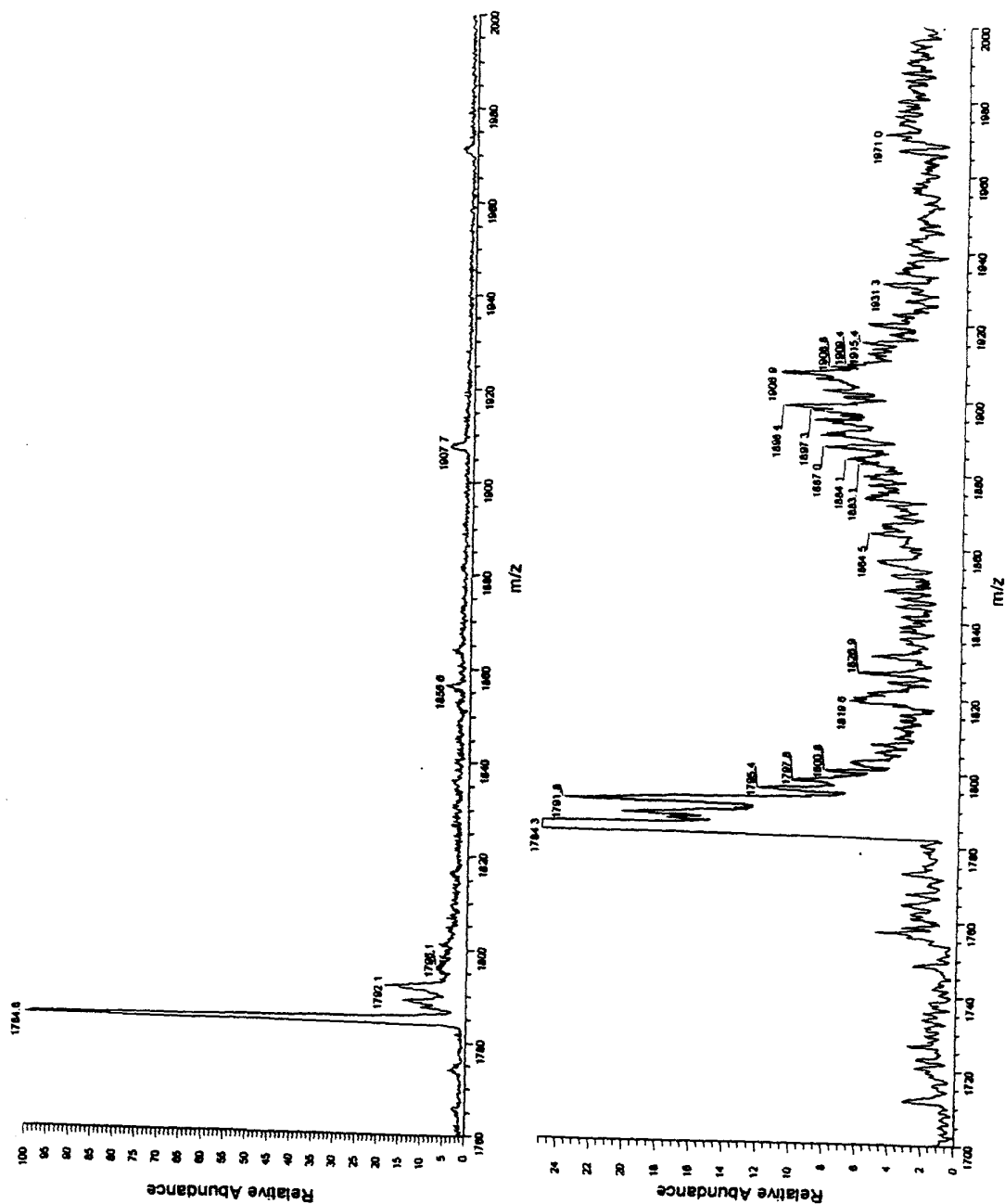
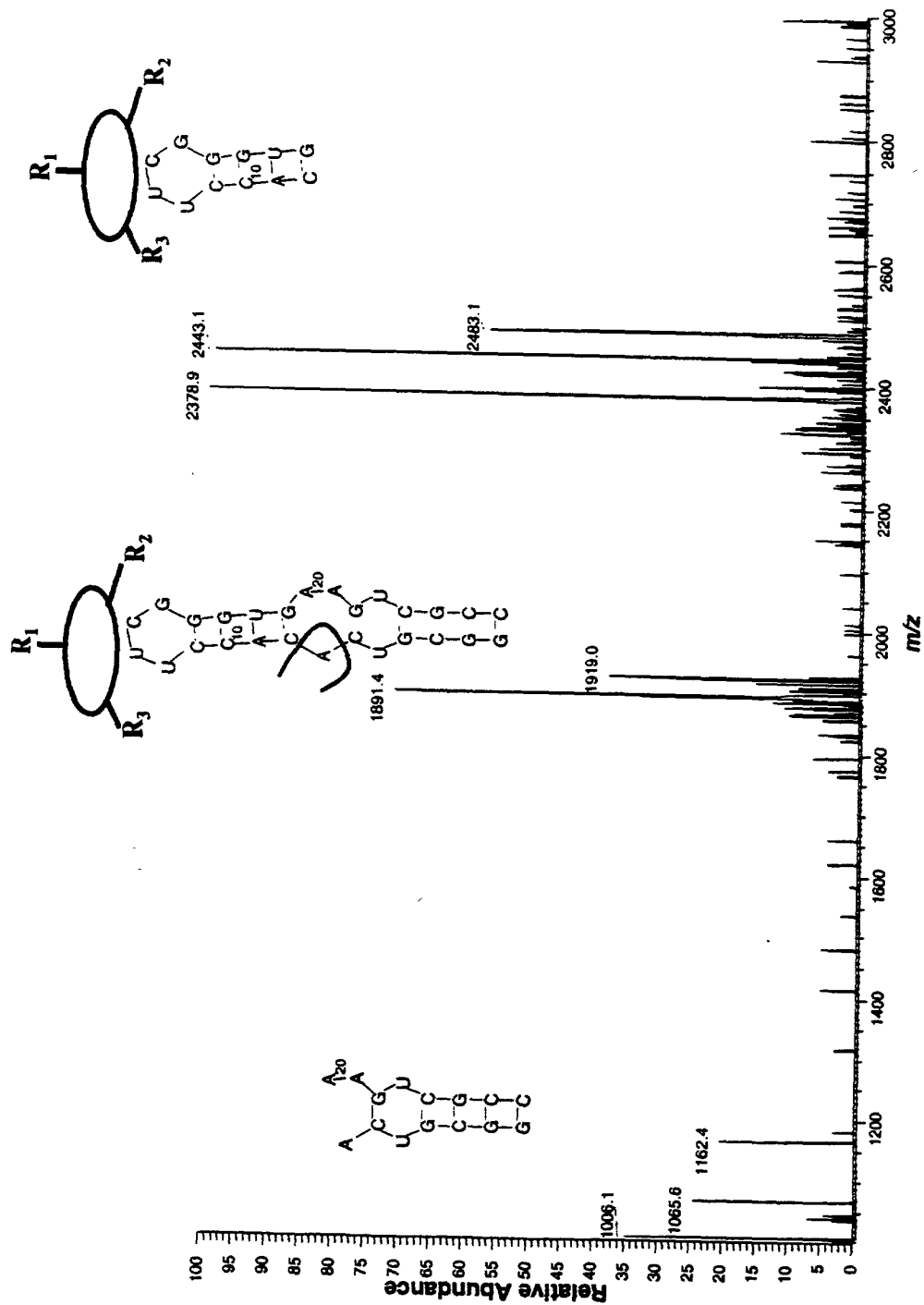


Figure 3. RNA/DNA chimera+paramomycin (upper); chimera+library (lower)



**Figure 4. MS-MS analysis of member bound to RNA/DNA chimera**

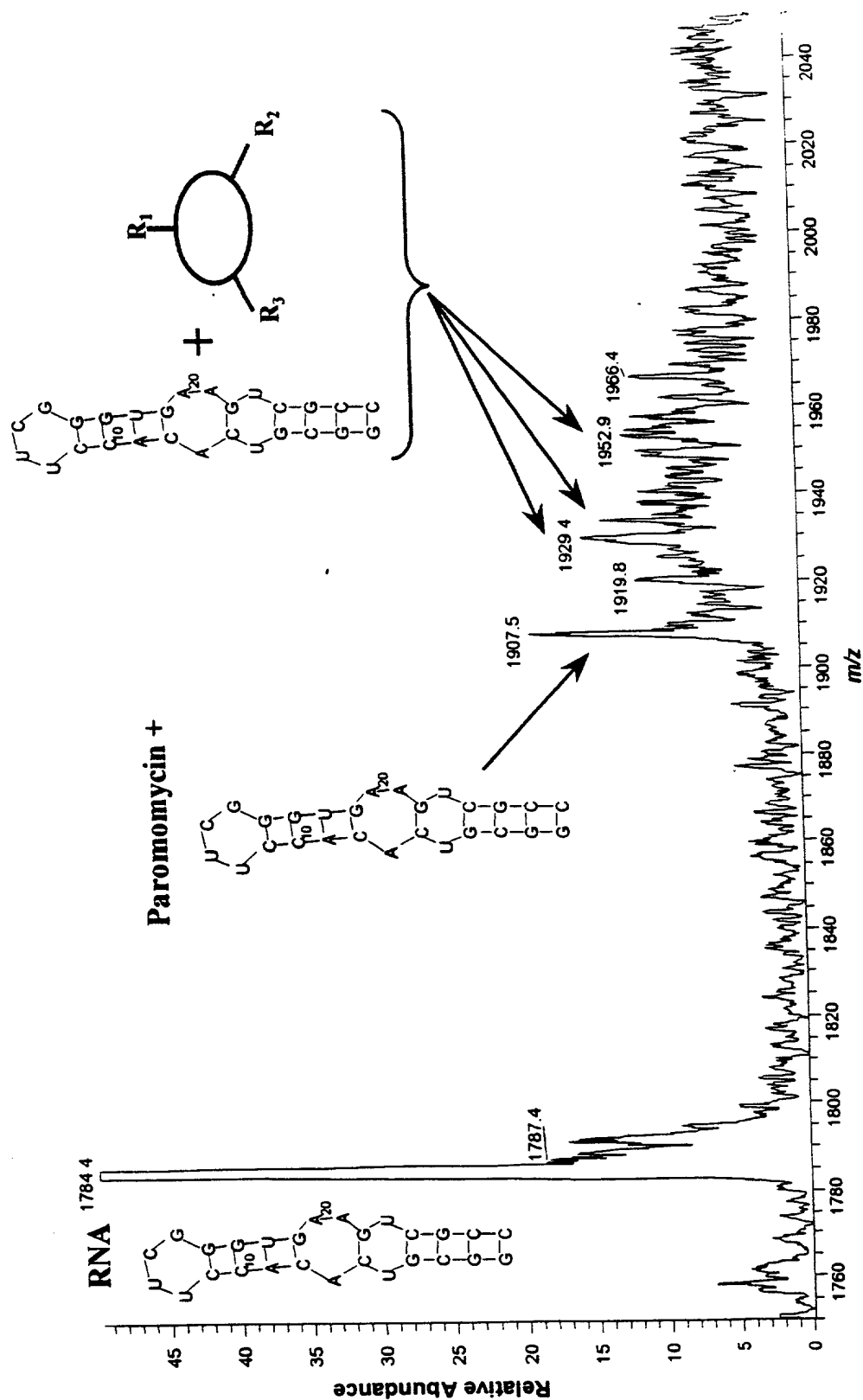


Figure 5. ESI-MS of RNA/DNA chimera bound to paromomycin and library

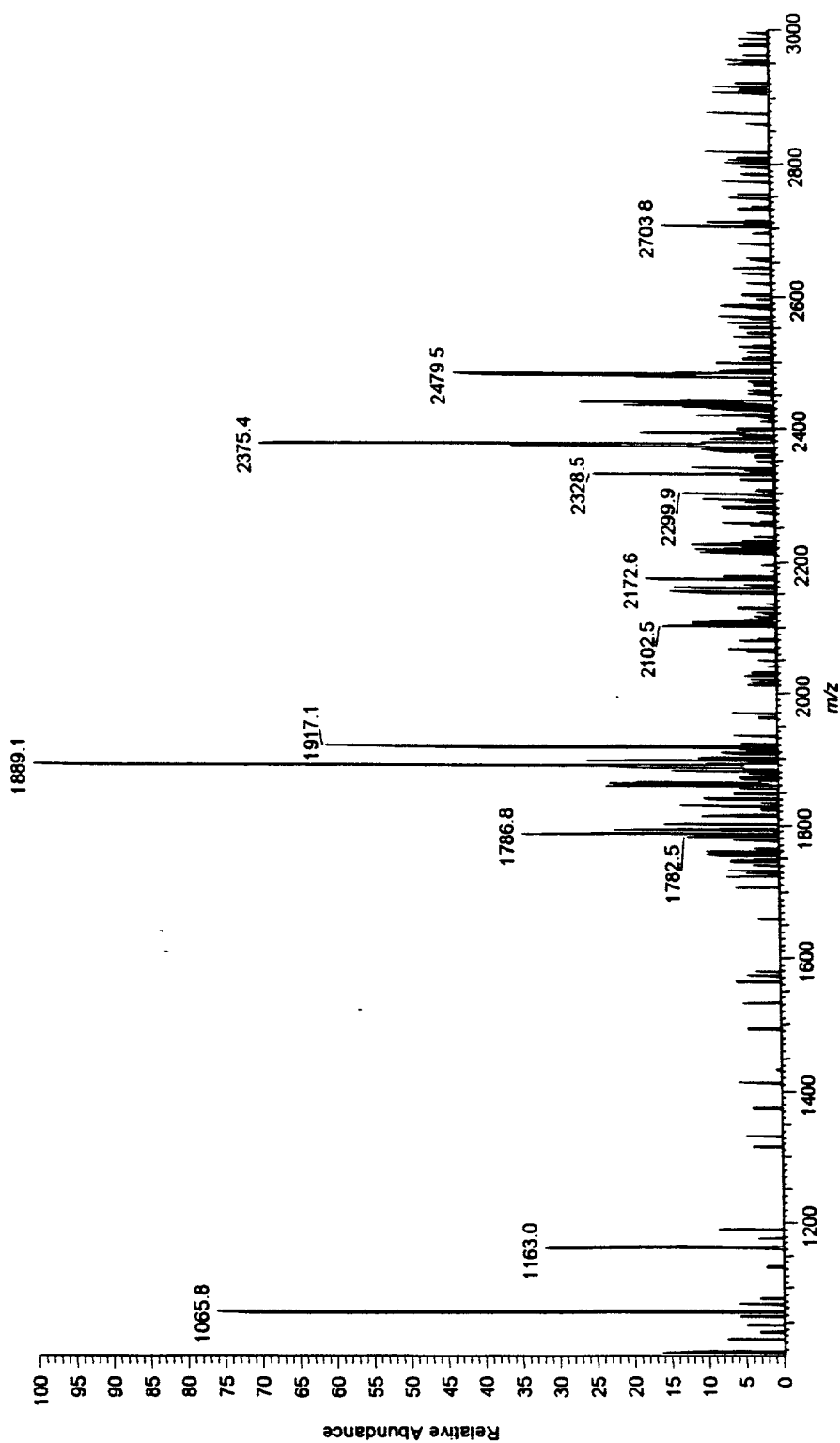


Figure 6. MS/MS of RNA/DNA chimera + compound with mass 665.1 not bound at the A-site



Figure 7. MS-MS analysis of member bound to RNA/DNA chimera at the A-Site





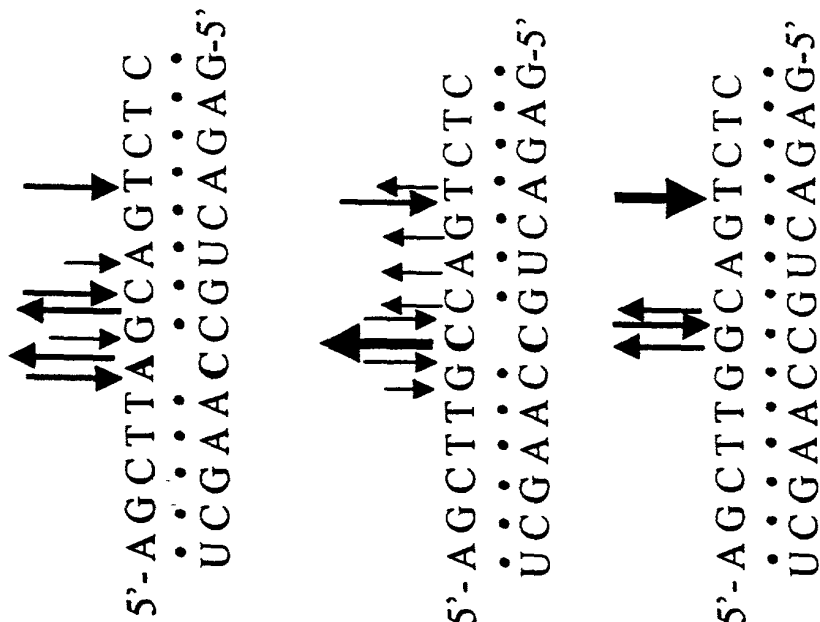
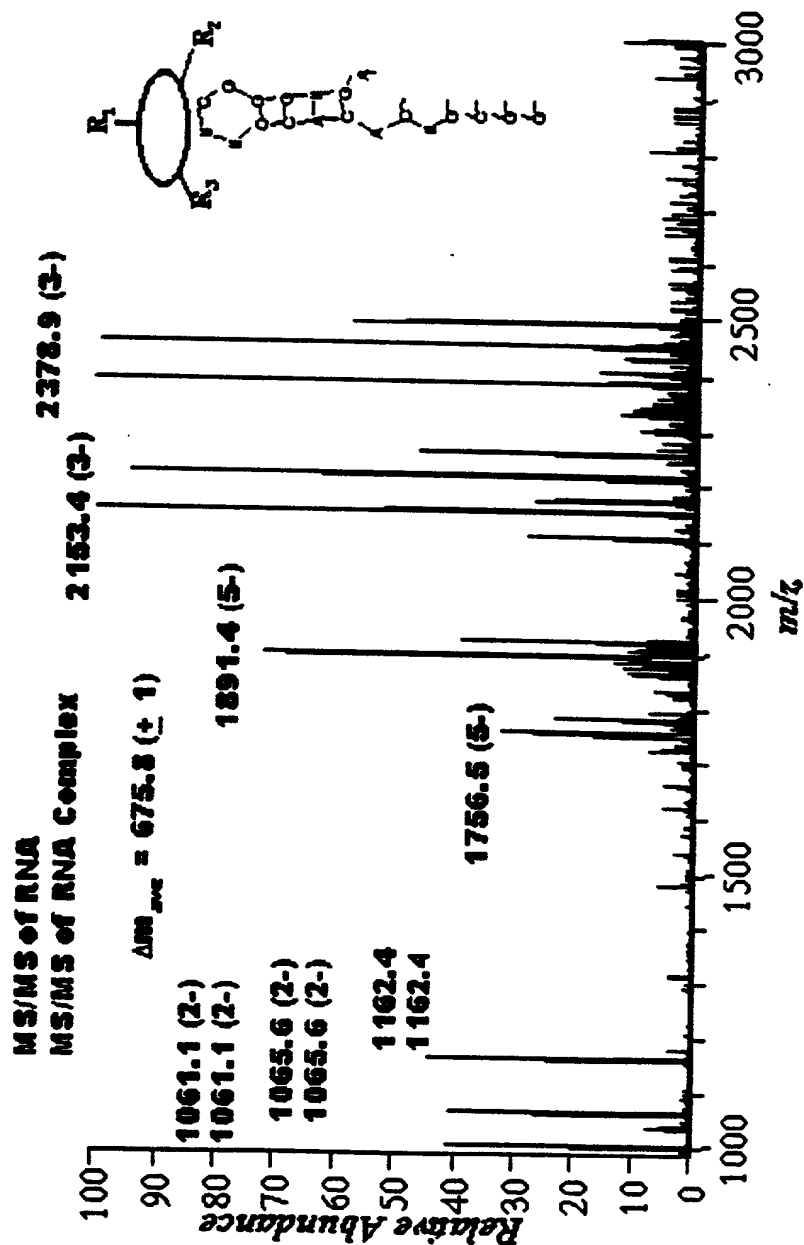


Figure 9. MS Fragmentation of DNA:RNA duplexes

# **MASS Analysis of Binding Location** **non-A site binder**

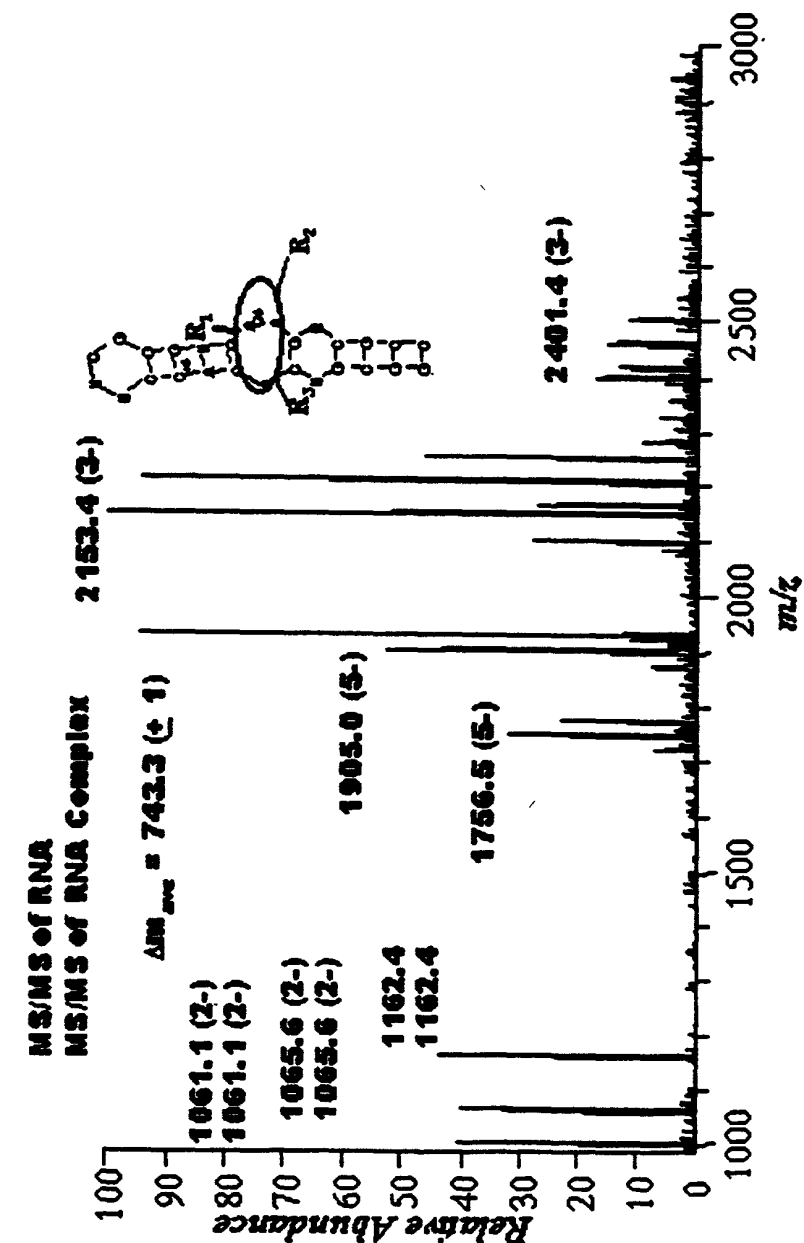
**FIGURE 10**



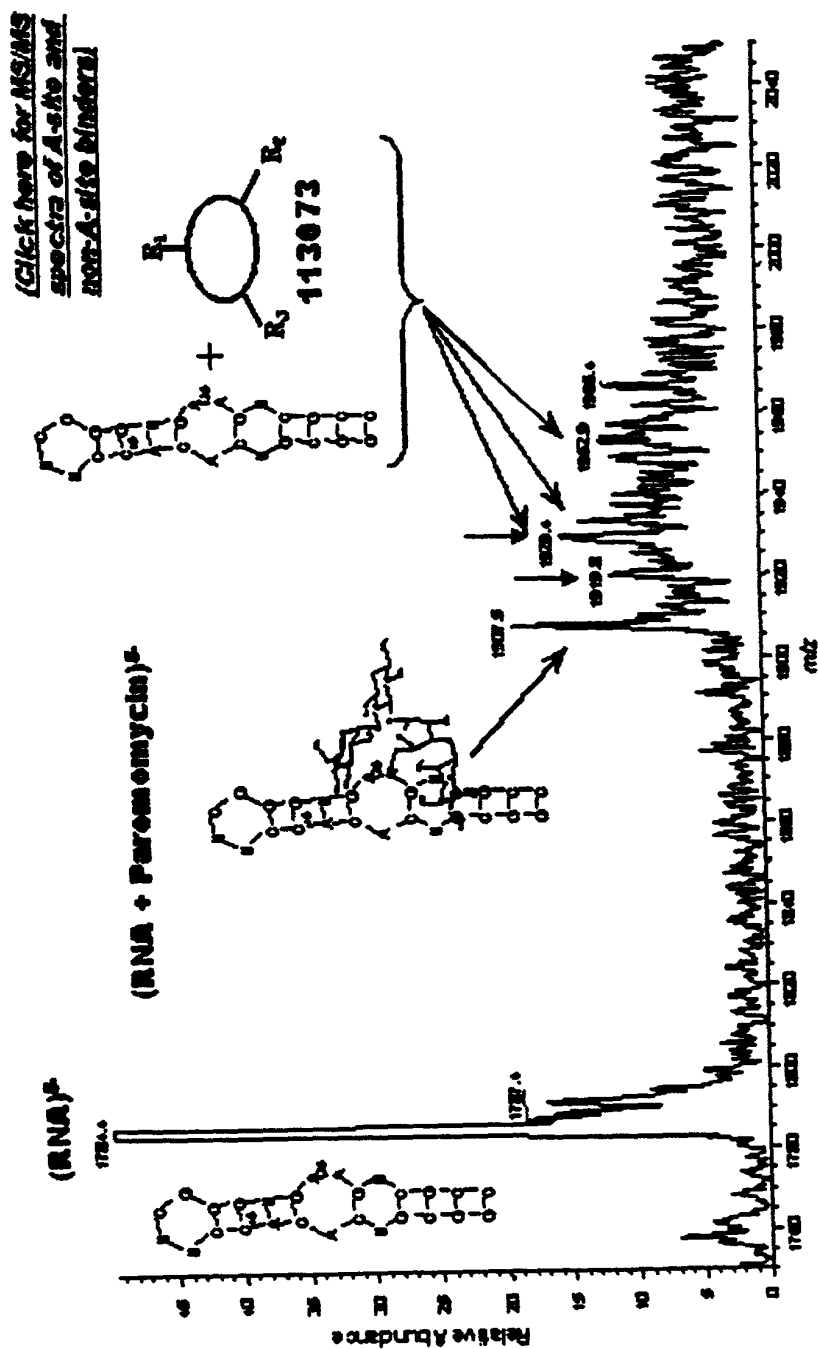
# **MASS Analysis of Binding Location**

non-A site binder

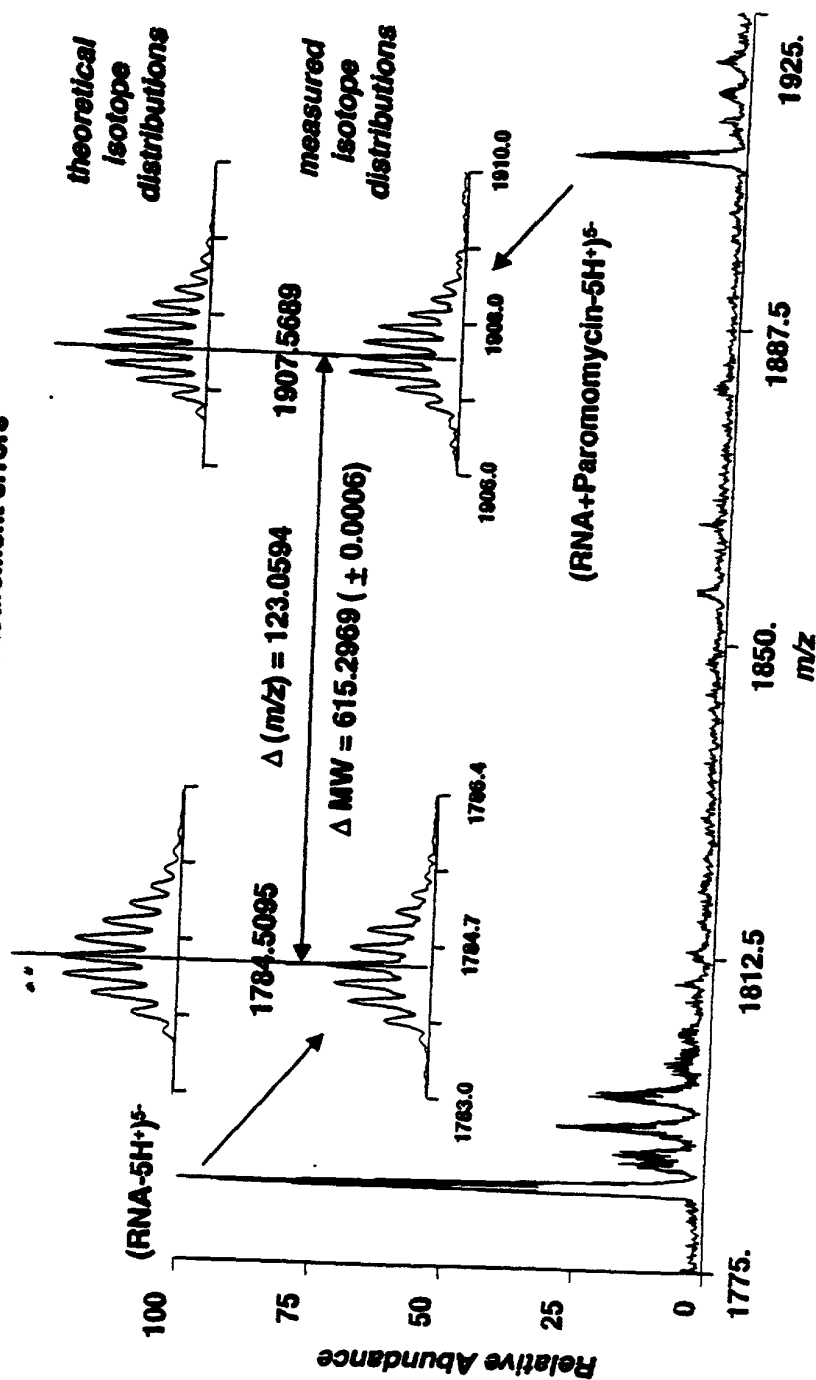
**FIGURE 11**



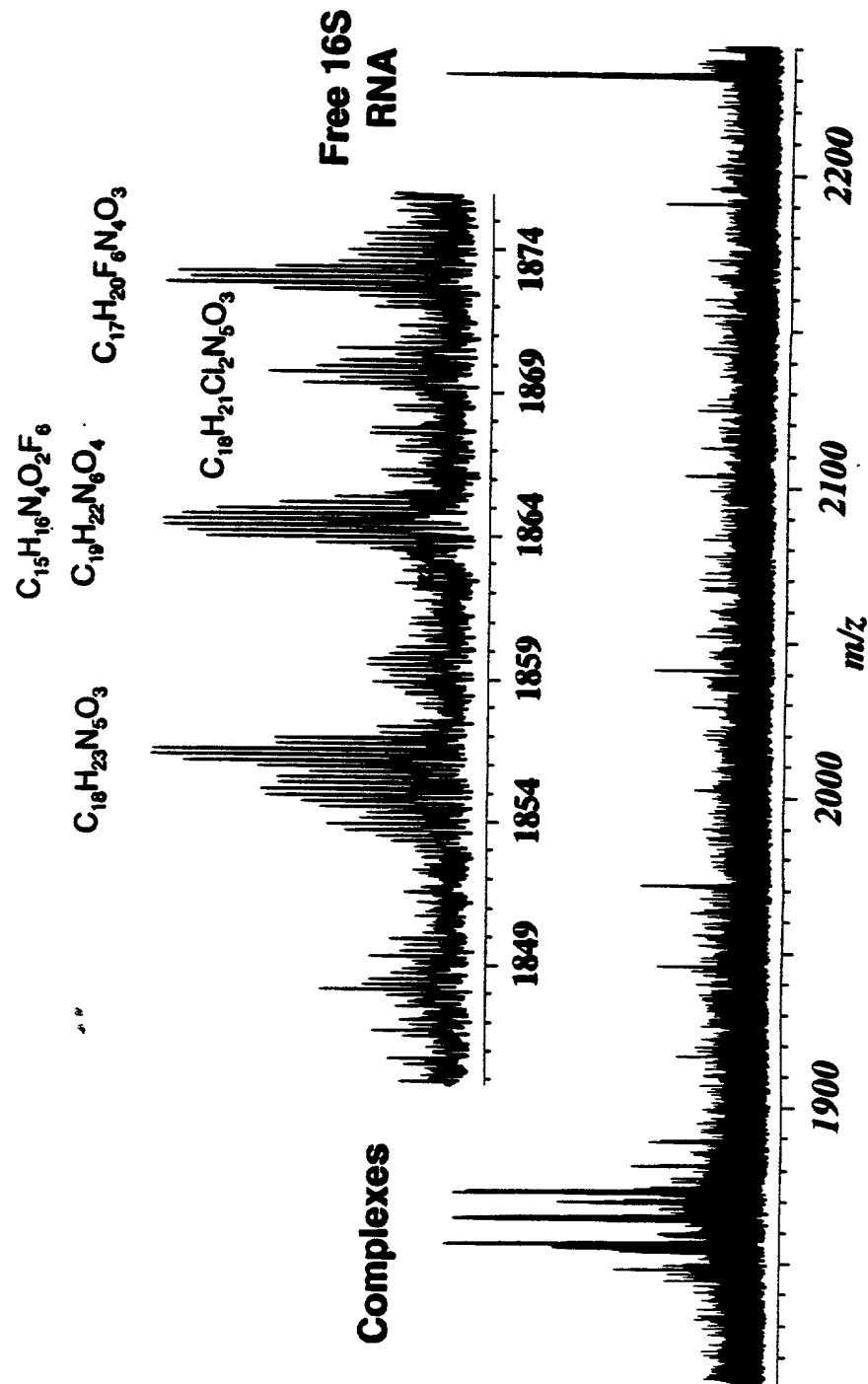
## FIGURE 12



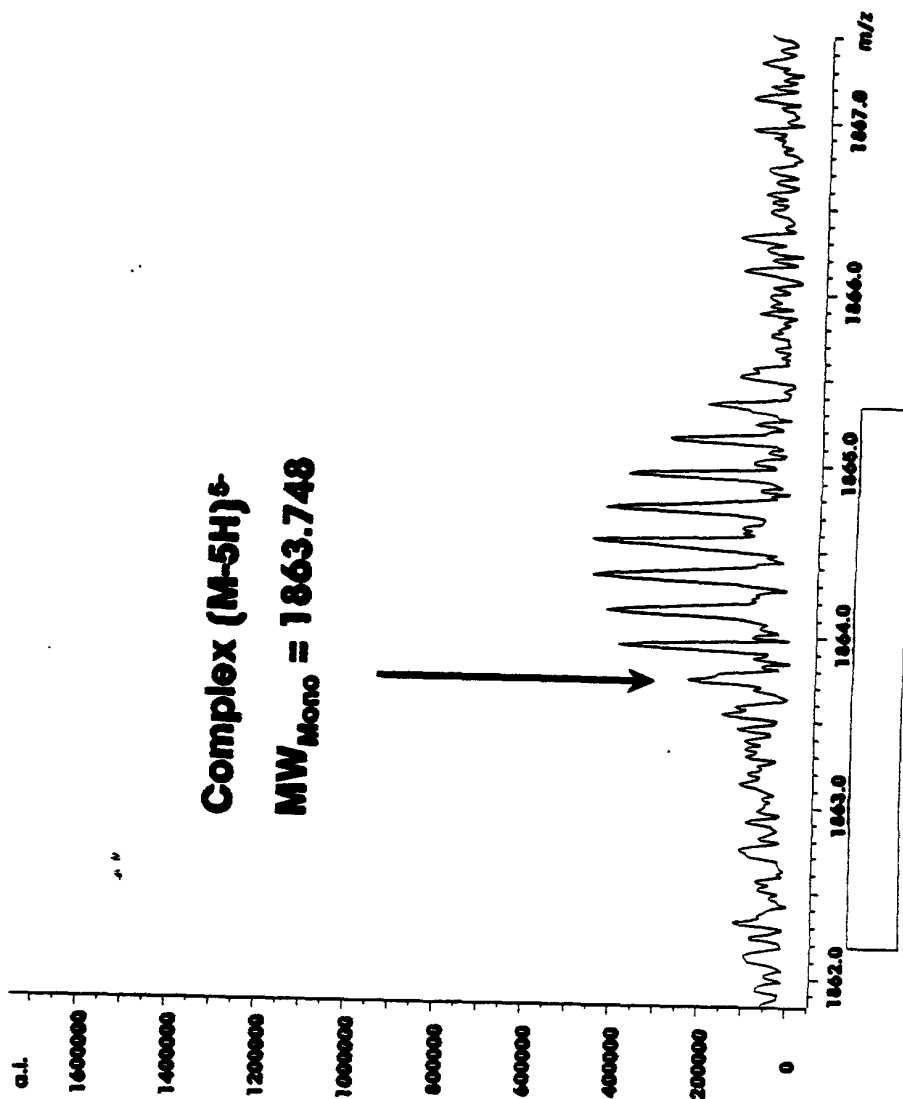
**Figure 13**  
**High Precision ESI-FTICR Mass Measurement of**  
**16S A site RNA/Paromomycin Complex**  
 use of unbound RNA as internal mass standard  
 provides low ppm mass measurement errors

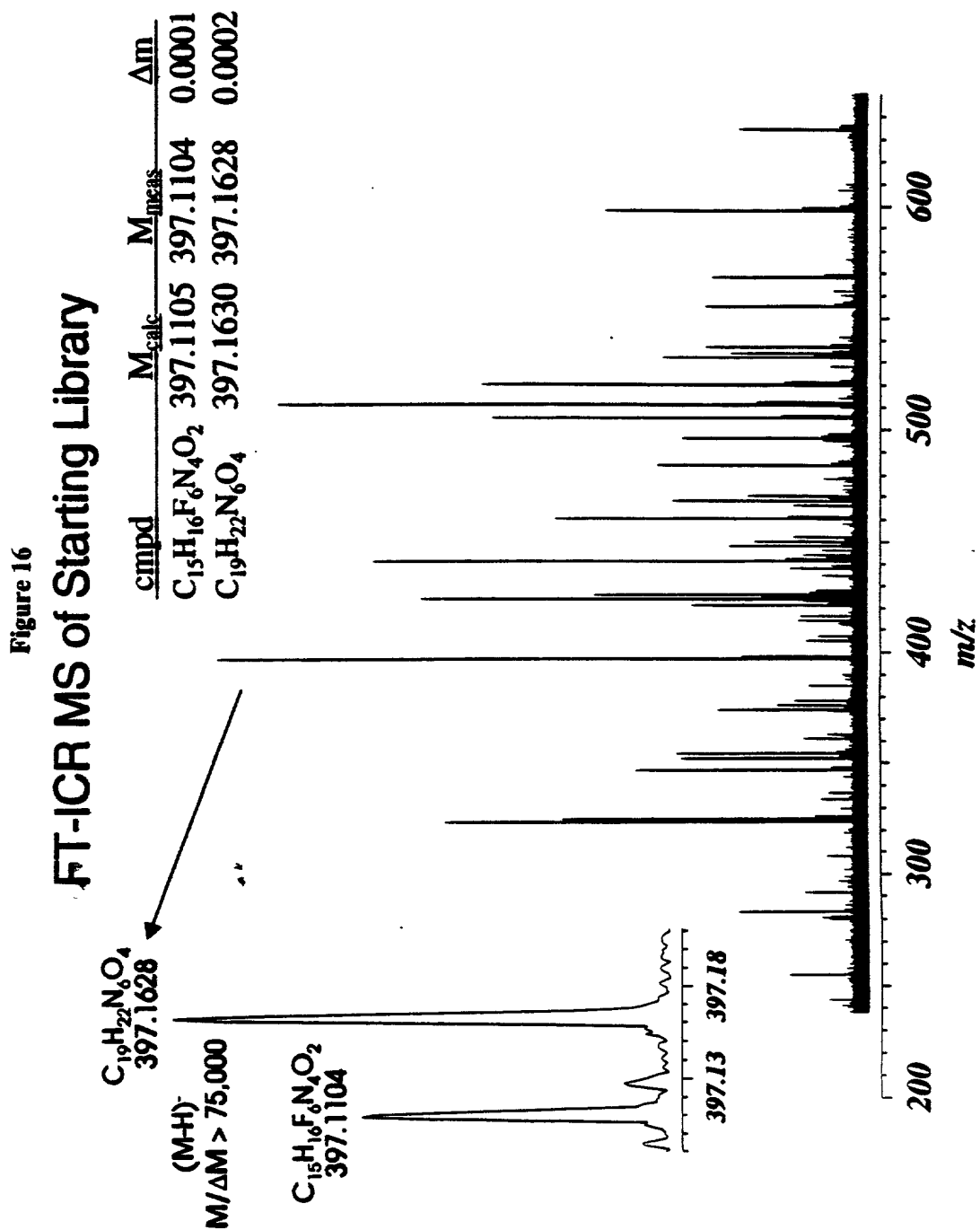


**Figure 14**  
**MASS of 60-Member Ibis Library Against 16S A-site RNA**



**Figure 15**  
**MASS of 60-member Library against 16S A-site Model**







**Figure 17**  
**Compound Identification from a 60-member**  
**Combinatorial library with MASS**

<b>Complex <math>M_{meas}</math></b>	<b>9320.300<math>\pm</math>.009 Da</b>
<b>RNA <math>M_{meas}</math></b>	<b>8922.189<math>\pm</math>.009</b>
<b><math>\Delta M</math></b>	<b>398.111<math>\pm</math>.009 Da</b>

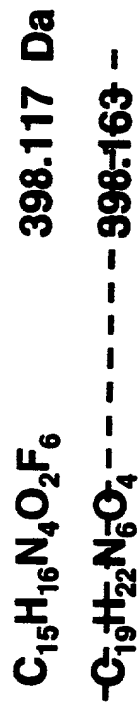


Figure 18

# Elemental Composition Constraints

Measured Mass: 615.2969

Mass Tolerance: 1.0 ppm

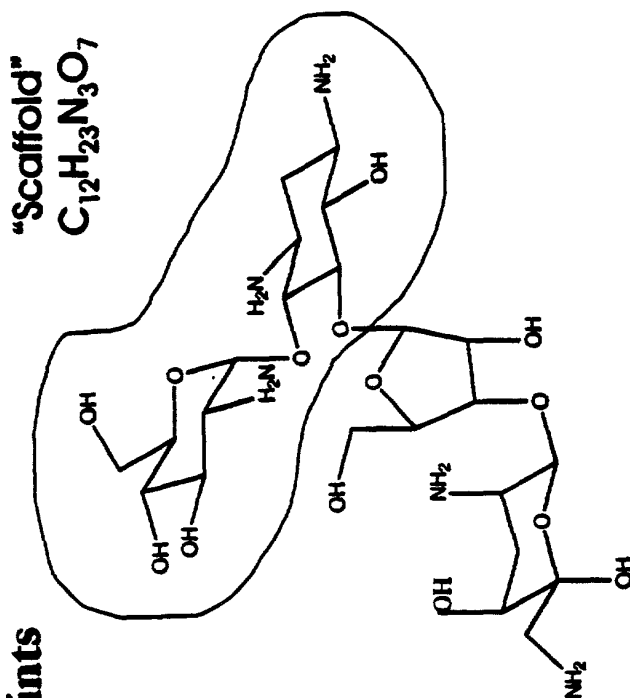
Charge: 0

Element	Min. atoms	Max. atoms
<sup>12</sup> C	12	30
<sup>1</sup> H	23	60
<sup>16</sup> O	7	20
<sup>14</sup> N	3	20

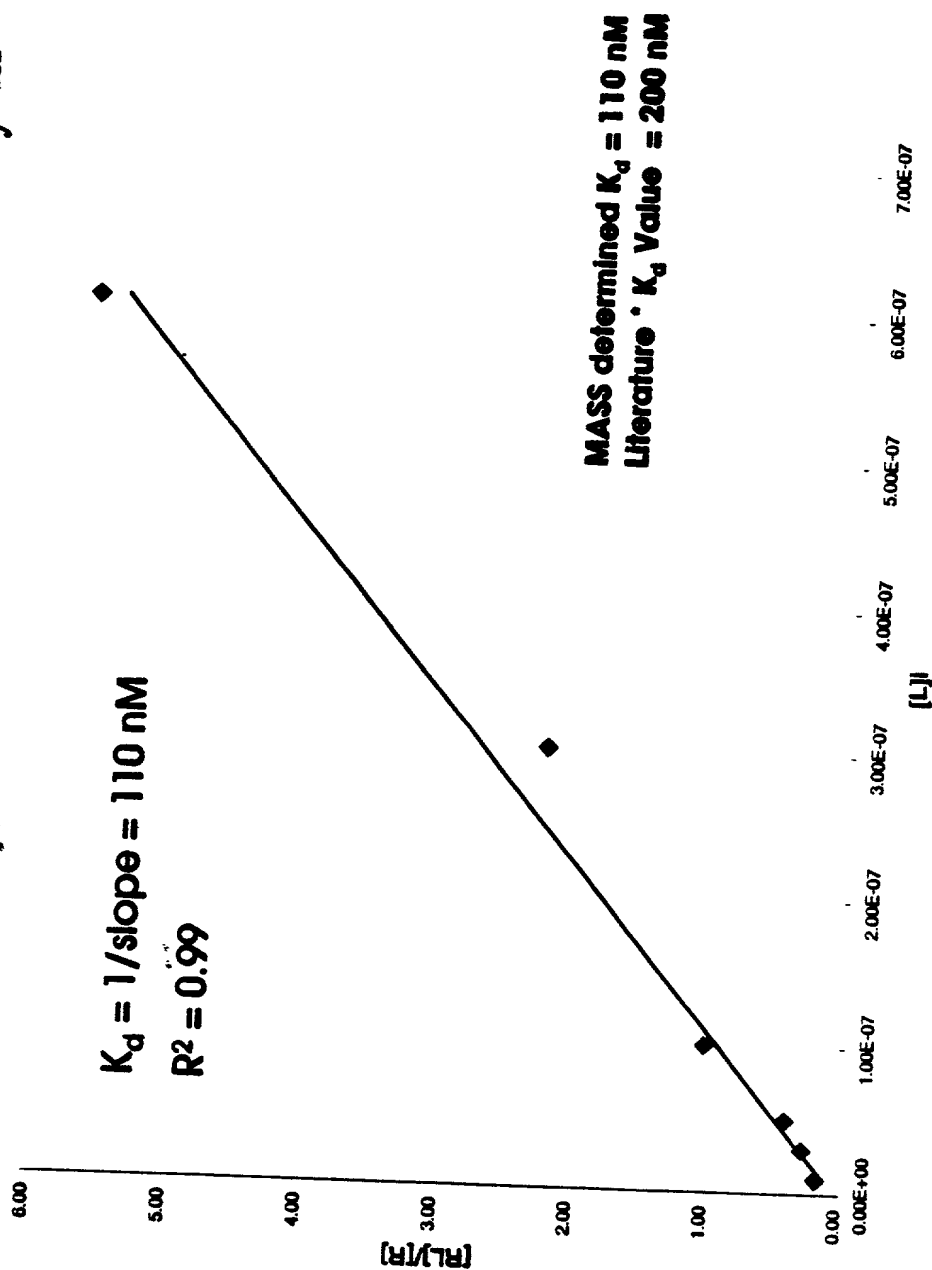
## Possible Elemental Compositions:

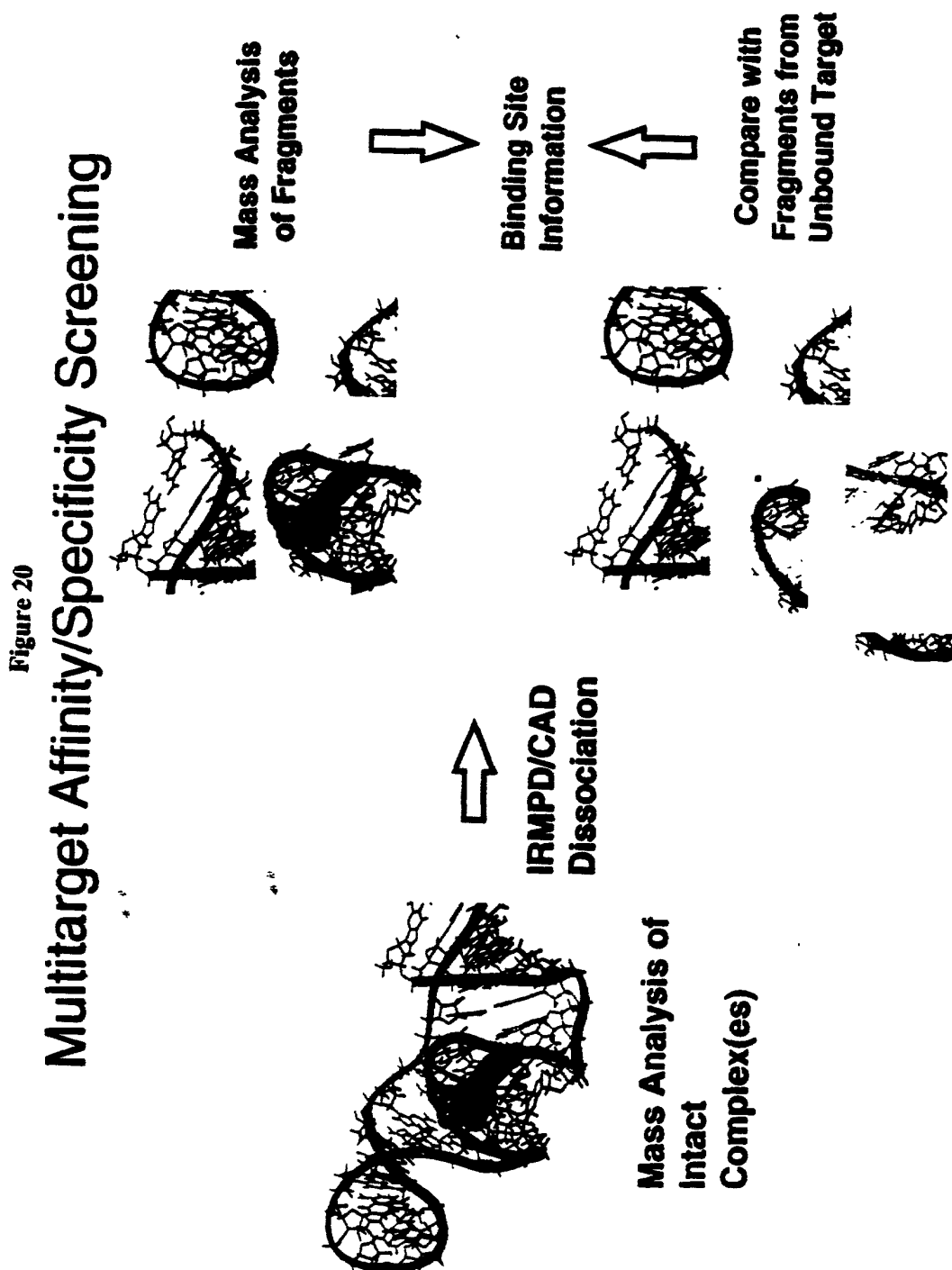
Calc. Mass (amu)	Error (ppm)	Molecular Formula
615.296291	0.98	<sup>16</sup> O <sub>4</sub> <sup>14</sup> N <sub>19</sub> <sup>12</sup> C <sub>21</sub> <sup>1</sup> H <sub>33</sub>
615.296298	0.98	<sup>16</sup> O <sub>9</sub> <sup>14</sup> N <sub>12</sub> <sup>12</sup> C <sub>22</sub> <sup>1</sup> H <sub>39</sub>
615.296305	0.97	<sup>16</sup> O <sub>14</sub> <sup>14</sup> N <sub>5</sub> <sup>12</sup> C <sub>23</sub> <sup>1</sup> H <sub>45</sub>
615.296808	0.15	<sup>16</sup> O <sub>15</sub> <sup>14</sup> N <sub>17</sub> <sup>12</sup> C <sub>8</sub> <sup>1</sup> H <sub>41</sub>
615.296815	0.14	<sup>16</sup> O <sub>20</sub> <sup>14</sup> N <sub>10</sub> <sup>12</sup> C <sub>9</sub> <sup>1</sup> H <sub>47</sub>

Further constrain by  
 elemental  
 composition of  
 "letters"  
 unintended  
 products...



**Figure 19**  
**MASS  $K_d$  determination for 16S-Paromomycin**





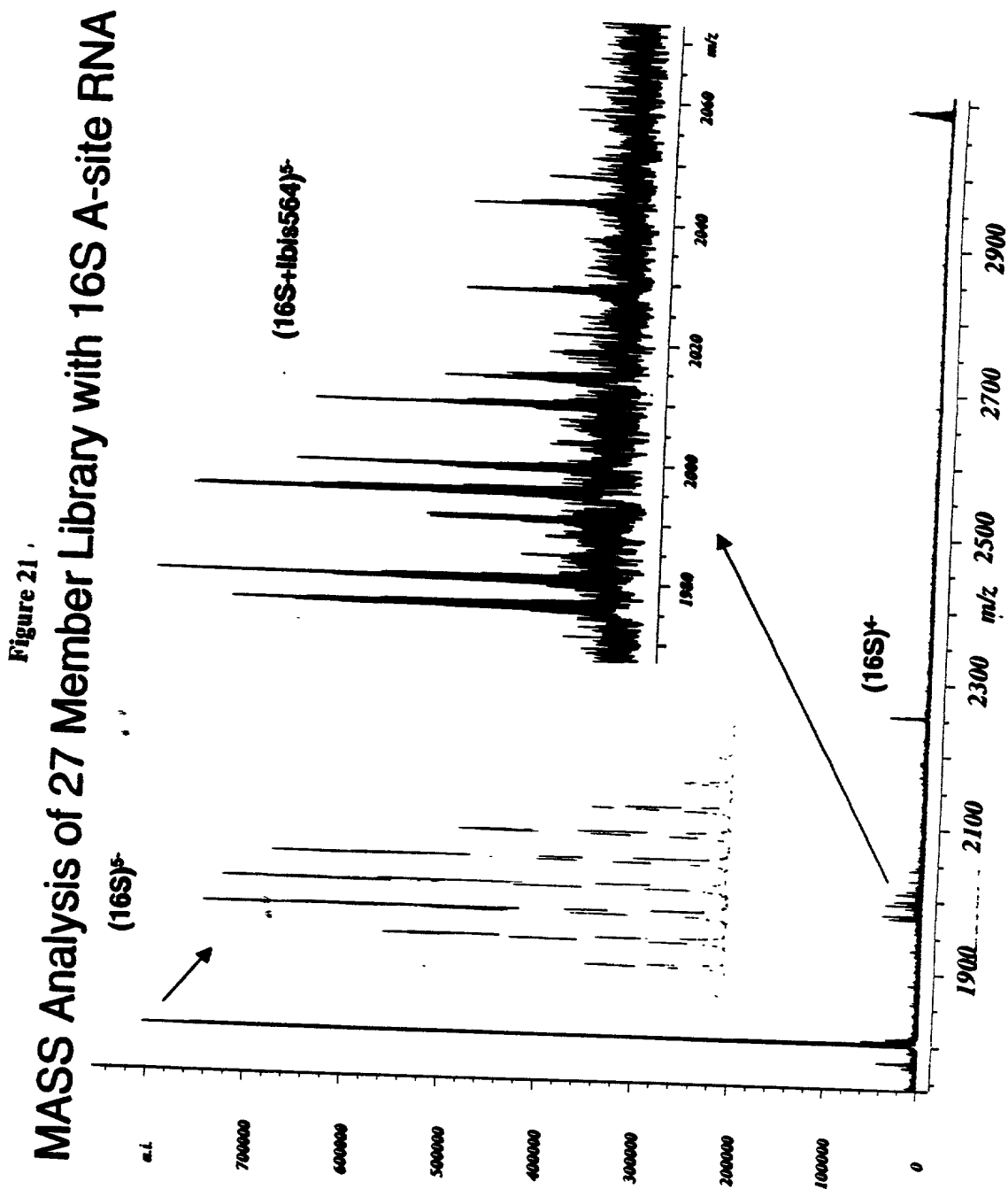


Figure 22

# MASS Protection Assay

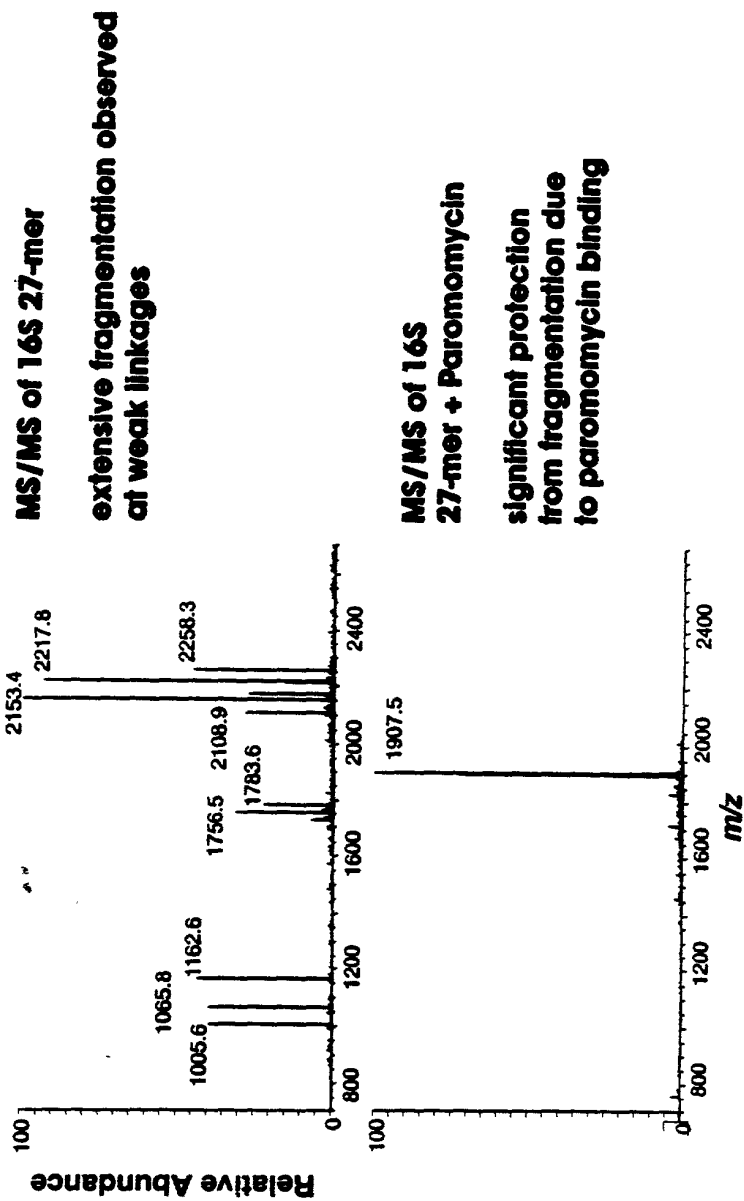
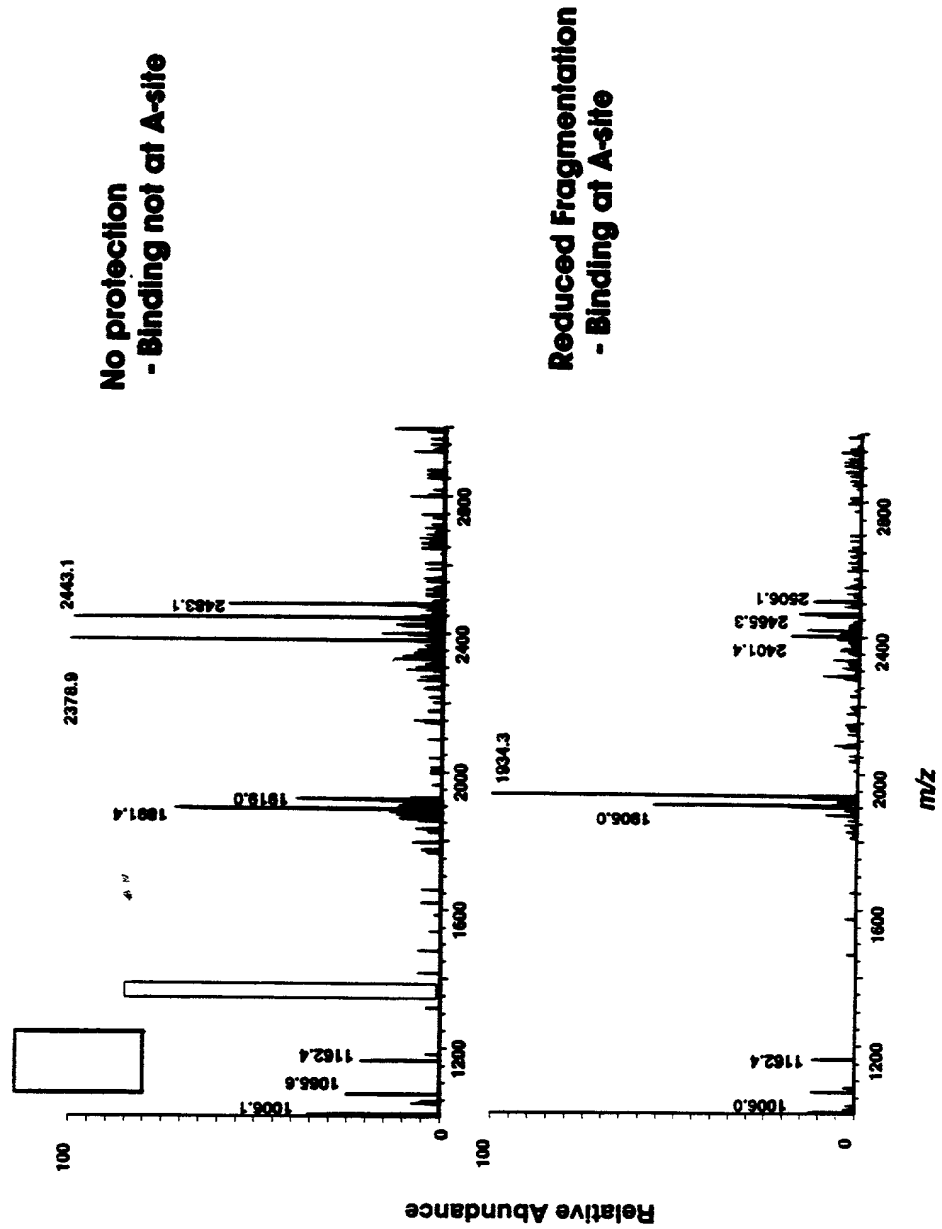
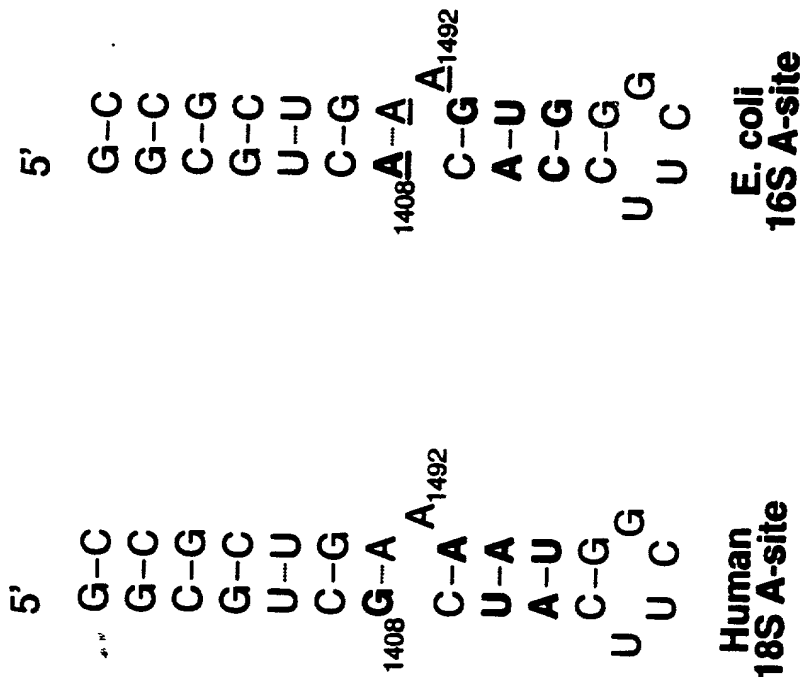


Figure 23  
**MASS Protection Assay**



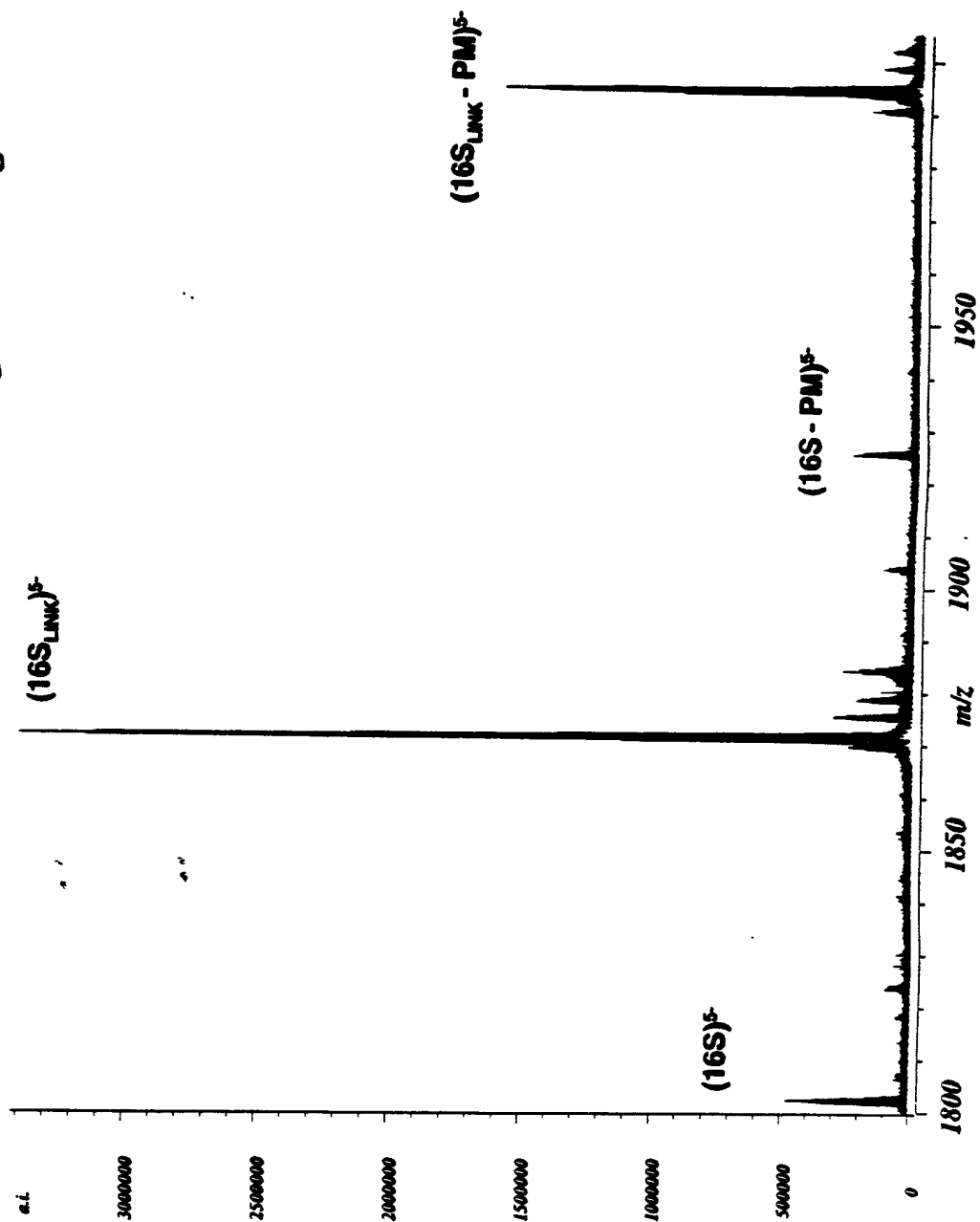
**Figure 24**  
**Eukaryotic and Prokaryotic A-Sites**  
 Aminoglycoside antibiotics bind to  
 A-site of decoding region in 16S RNA



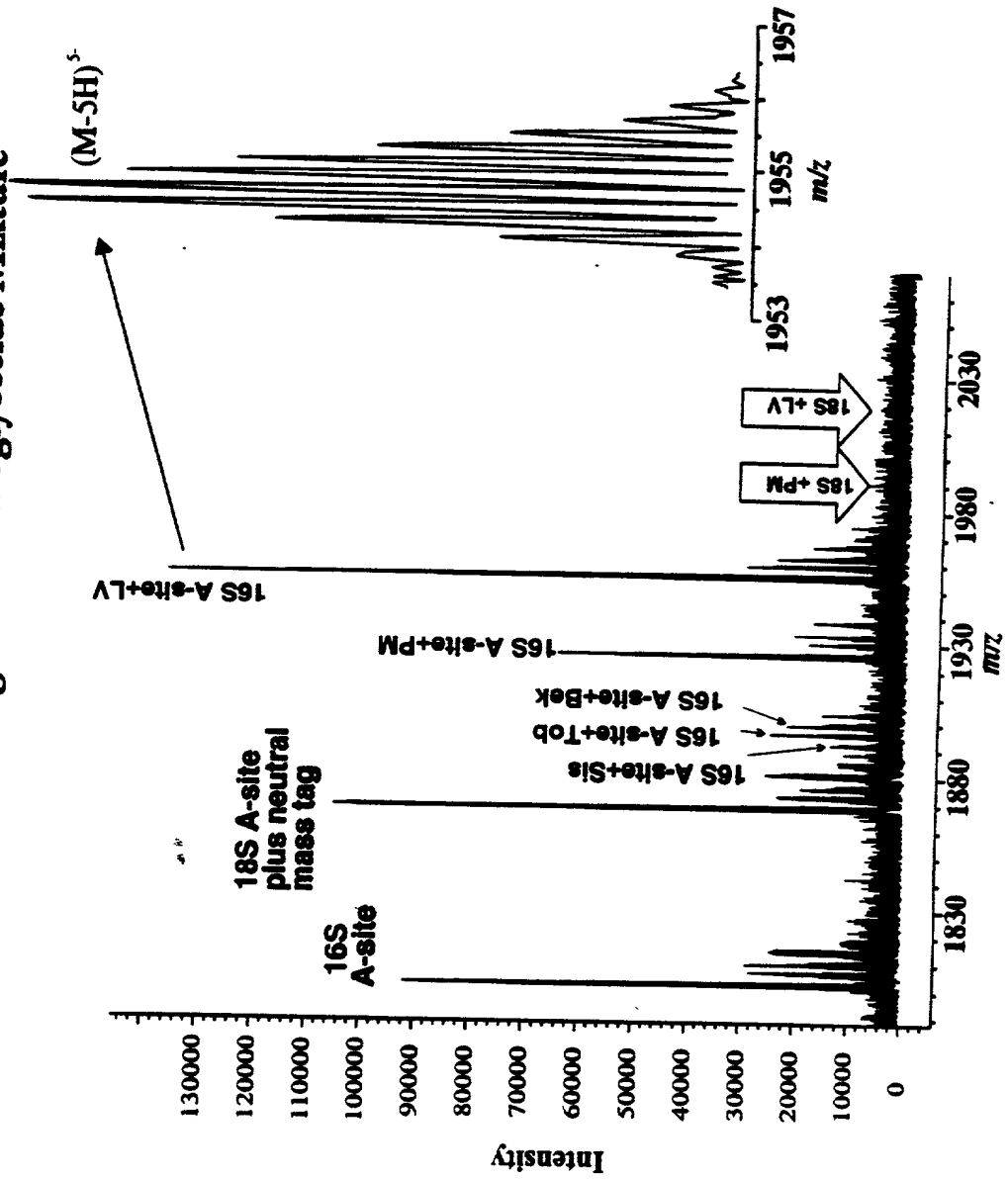
$\Delta$  MW = 15.011 Da



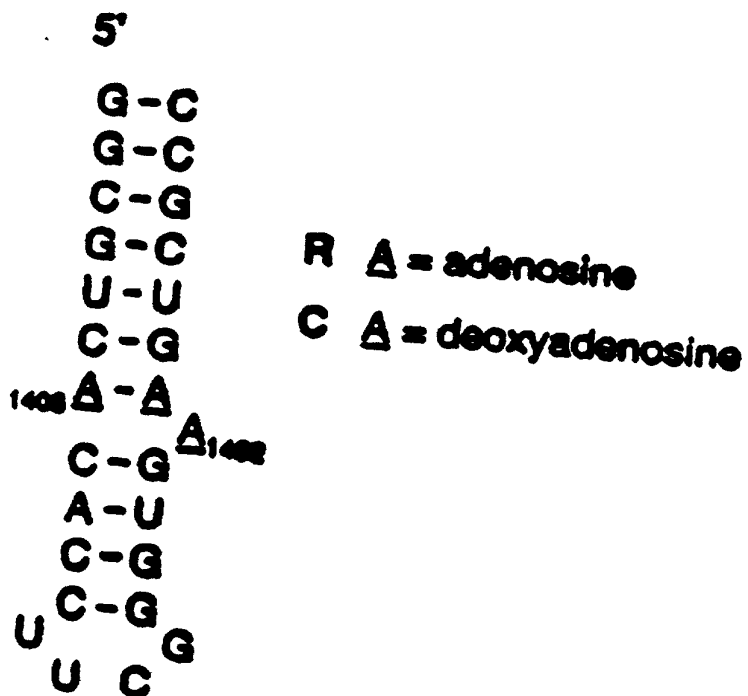
Figure 25  
Neutral Mass Tag Does Not Affect Ligand Binding



**Figure 26**  
**Simultaneous Screening of 16S A-site and 18S A-site**  
**Model RNAs Against Aminoglycoside Mixture**



**Figure 27**



00884347 061901  
 T06T50 / FE48860

Figure 28

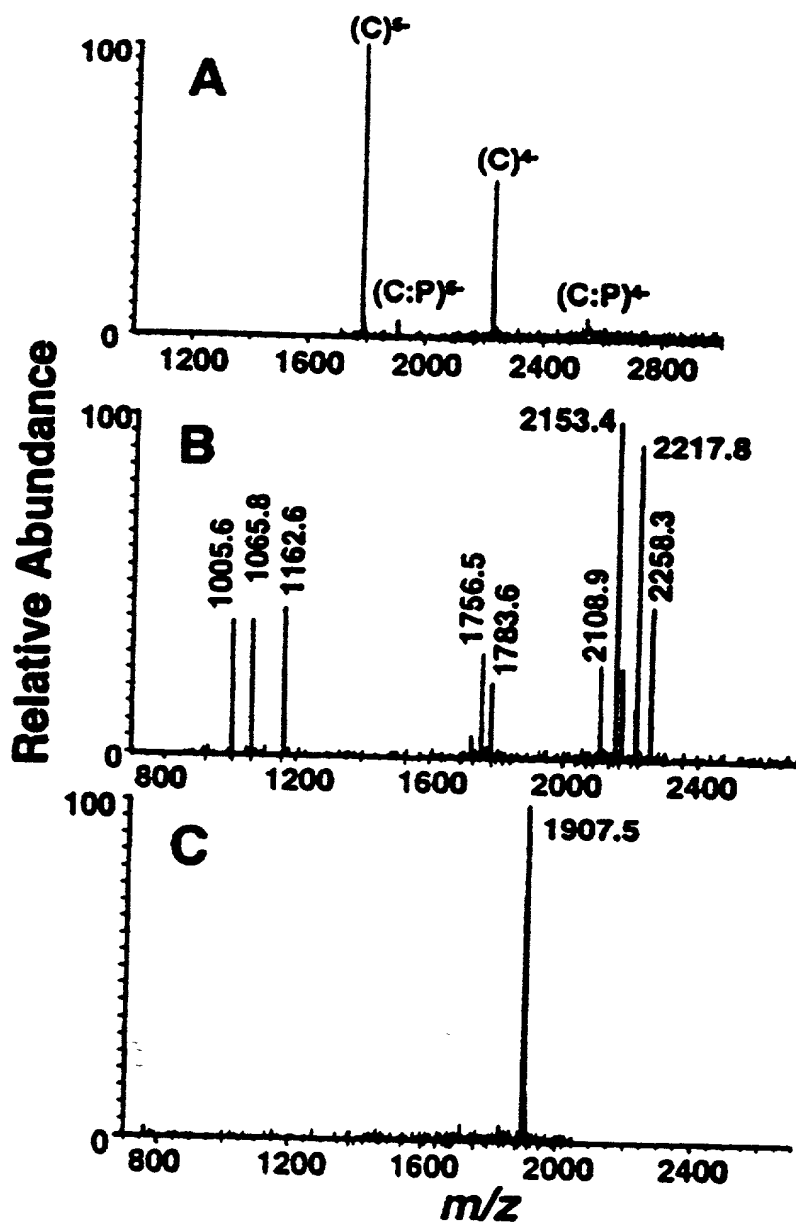


Figure 29

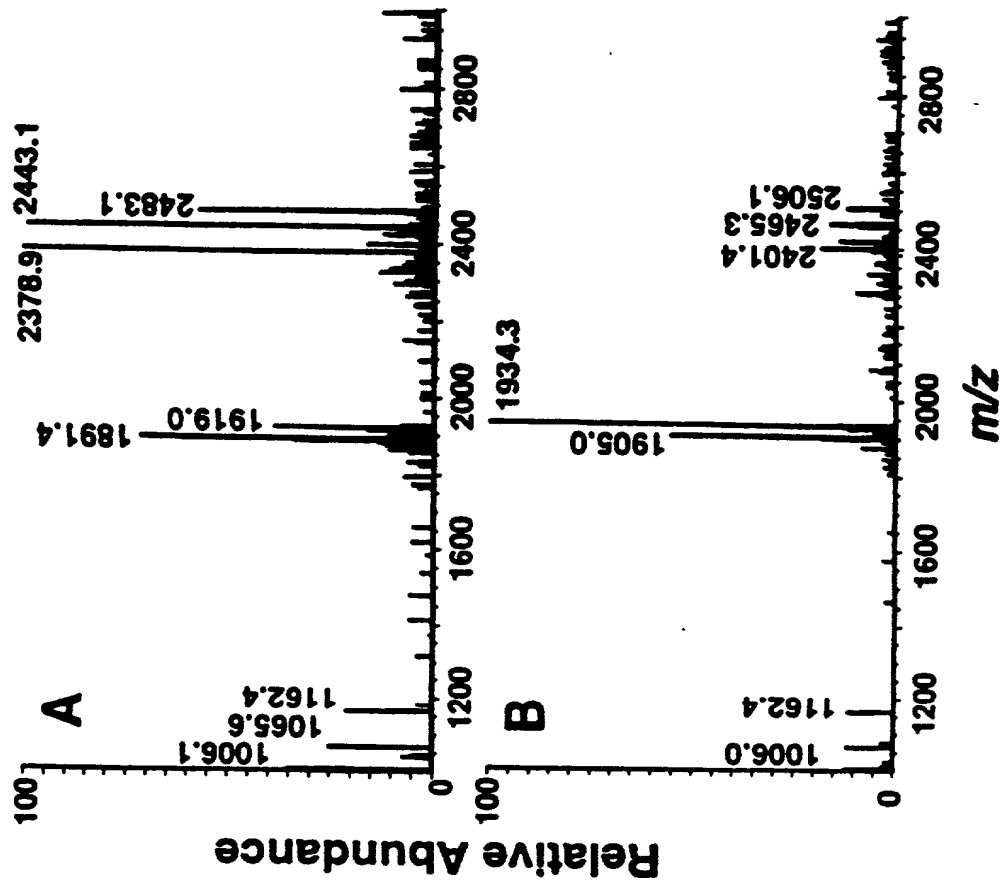


Figure 30

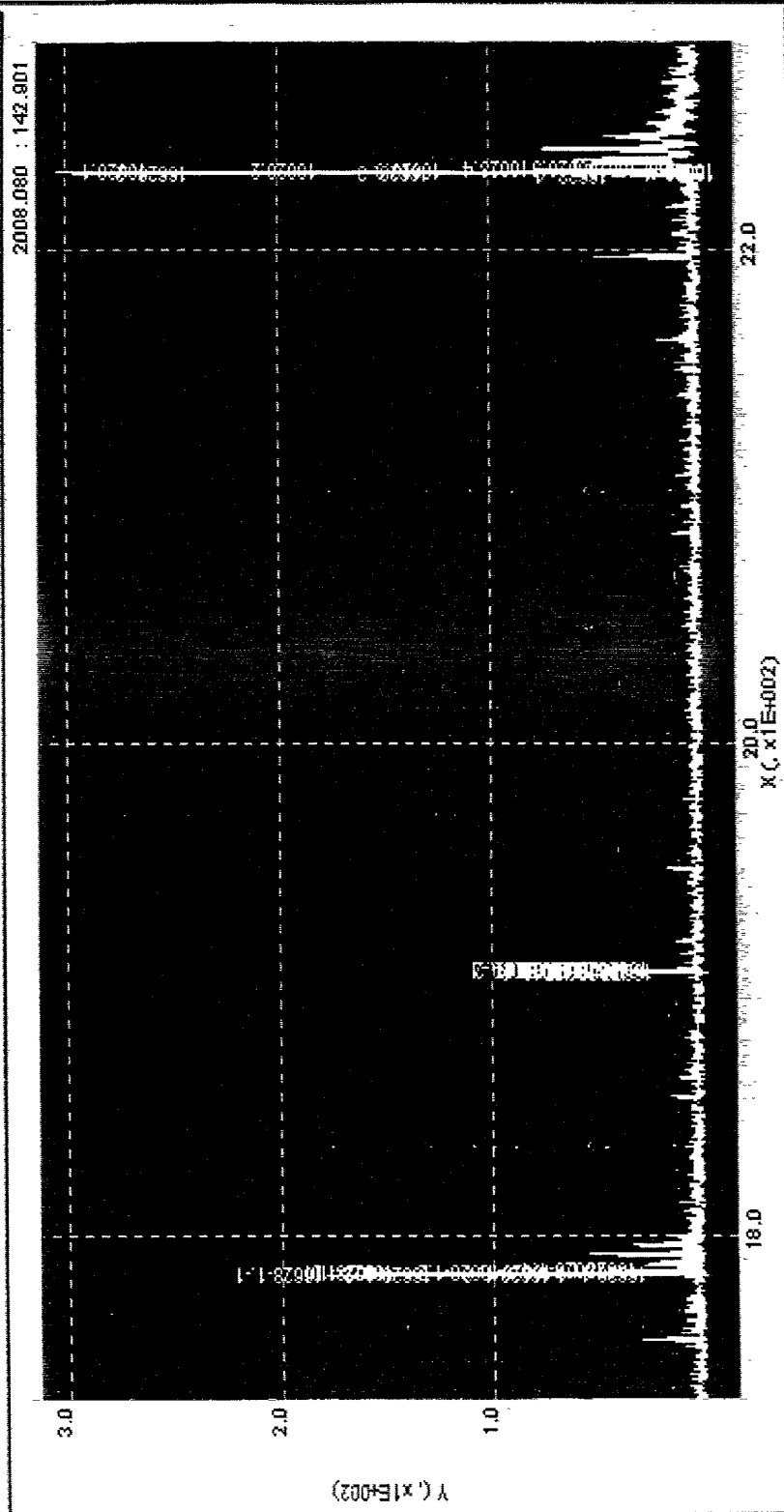


Figure 31

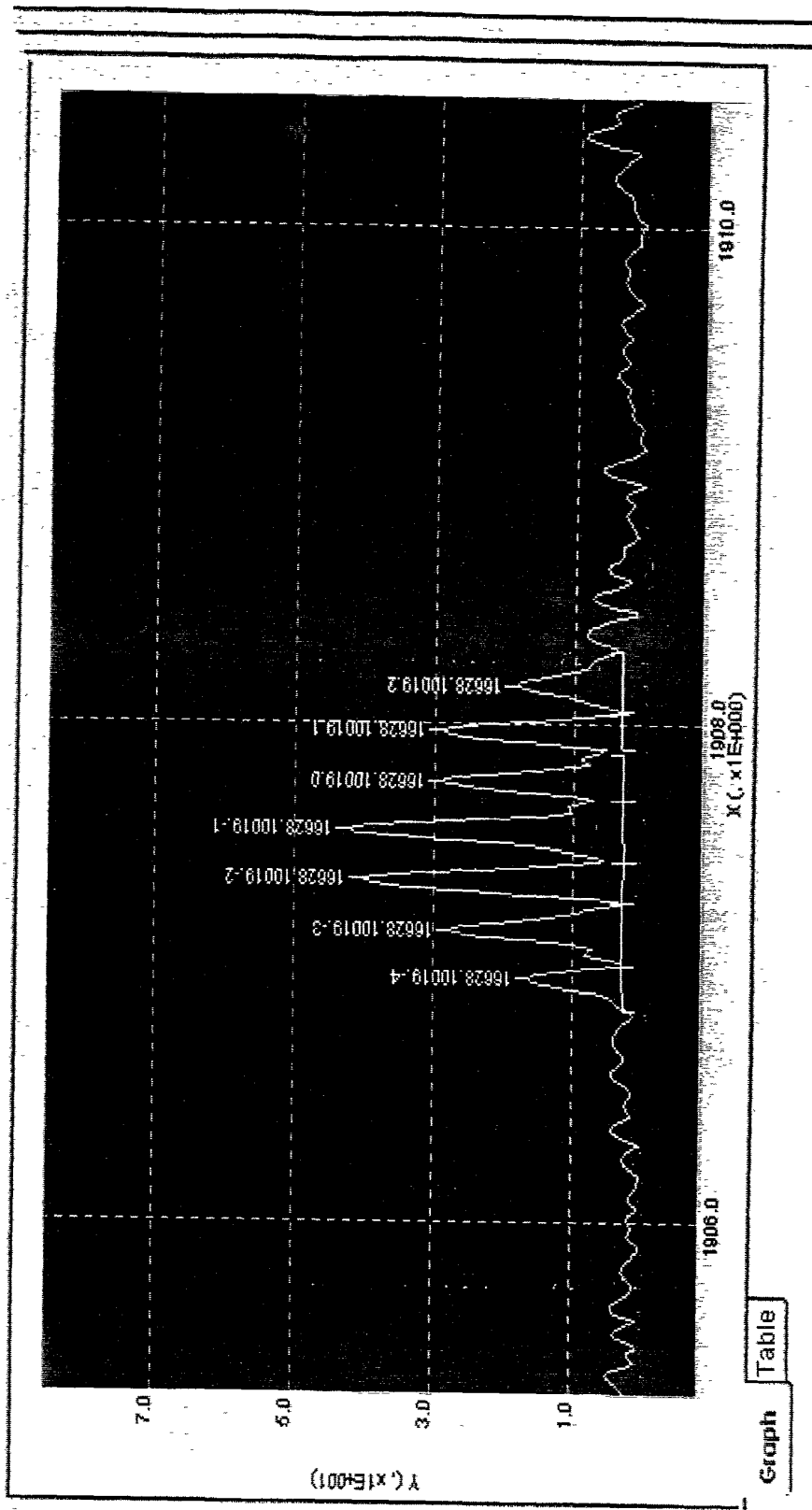


Figure 32

nr	name	apex	start	stop	height	area
1	16628-1.4	1783.710	1783.635	1783.834	14.55	1.63
2	16628-1.3	1783.909	1783.834	1783.972	60.04	5.15
3	16628-1.2	1784.109	1784.021	1784.184	115.60	11.14
4	16628-1.1	1784.308	1784.233	1784.383	167.34	15.89
5	16628-1.0	1784.508	1784.433	1784.620	133.94	14.74
6	16628-1.1	1784.707	1784.620	1784.795	136.60	13.38
7	16628-1.2	1784.907	1784.795	1784.982	82.63	8.56
8	16628-1.3	1785.107	1785.032	1785.219	57.81	5.21
9	16628-1.4	1785.306	1785.232	1785.369	32.31	2.65
10	16628-1.5	1785.506	1785.456	1785.569	17.67	1.12
11	16628-10019.4	1906.974	1906.874	1907.031	12.63	1.00
12	16628-10019.3	1907.173	1907.045	1907.273	22.54	2.11
13	16628-10019.2	1907.373	1907.287	1907.444	33.86	2.91
14	16628-10019.1	1907.572	1907.458	1907.701	34.87	3.30
15	16628-10019.0	1907.772	1907.701	1907.843	20.93	1.55
16	16628-10019.1	1907.972	1907.900	1908.043	21.03	1.55
17	16628-10019.2	1908.157	1908.086	1908.271	10.97	0.90
18	16628.4	2229.874	2229.679	2230.029	27.51	4.87
19	16628.3	2230.146	2230.029	2230.263	111.72	16.23
20	16628.2	2230.380	2230.263	2230.516	225.18	32.39
21	16628.1	2230.633	2230.516	2230.770	280.66	40.90
22	16628.0	2230.887	2230.770	2231.023	287.24	41.95
23	16628.1	2231.140	2231.023	2231.257	242.23	34.17

Graph Table



Figure 33

